Treatments for Improving Seed Germination in Eggplant and Related Species

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Keywords: BAP, dormancy, Fluridone, gibberellins, *Solanum*

Abstract

Orthodox seeds may present differences in germination due to several reasons. Poor seed germination limits the use of different species for culture or plant breeding. In this work, we tested treatments for improving germination in two cultivars of common eggplant (*Solanum melongena*) and in six accessions of the related species *S. macrocarpon*, *S. aethiopicum*, and *S. incanum*. With the exception of *S. incanum* MM-557, which did not germinate, seeds of the other accessions directly had lower final germination percentages when sown in Petri dishes with deep filter paper than those that were pretreated by surface sterilization and sown in nutrient medium; a further increase in the final germination percentages was achieved by addition of 1 mg L^{-1} GA_3 to the medium. The GA_3-pretreatment not only increased the final germination percentages but promoted germination also by reducing germination time. Other treatments included stratification of *S. macrocarpon* and *S. aethiopicum* seeds at 4°C for 15 or 30 days, as well as addition of 1 mg L^{-1} of TDZ and BAP for the BBS-168 accession, but this did not improve germination. The addition of 0.3 mg L^{-1} fluridone, an inhibitor of carotenoid biosynthesis, that is known to prevent ABA biosynthesis, increased the final germination percentages of *S. melongena*, *S. aethiopicum* and *S. macrocarpon* to a similar degree as GA_3. Seeds of *S. incanum* MM-557 did not respond to this treatment but the hybrid derived from this *S. incanum* accession with eggplant accession ANS-26 showed high germination percentages (70%, 13 days after sowing) in NM medium with GA_3, fluridone or both components. These results show that seed treatments can contribute to improving germination of *Solanum* accessions with low germination.

INTRODUCTION

Vegetable crops with orthodox seeds may present differences in germination due to possible dormancy, loss of viability during the conservation conditions, genetic differences among the materials, or aging. Seed dormancy is a condition of plant seeds that prevents germinating under optimal environmental conditions. Dormancy and germination are determined by the co-action of the growth potential of the embryo and the restraints imposed by the tissues surrounding it. In nature, dormancy is a mechanism that allows a number of species to survive in particular environments and regulates the time and place of germination to be most favorable for survival.

When seeds ripen they may be dormant (primary dormancy) or not dormant, that is, they germinate readily when given the proper environmental conditions of water, temperature, and/or other factors. However, seeds that do not have primary dormancy often acquire secondary dormancy as they dry.

There are three major mechanisms for imposing dormancy: (1) seed coverings that restrict water uptake, embryo expansion, gas permeability, leaching of inhibitors; (2) chemical inhibitors including several growth regulators and (3) morphological aspects such as small or undeveloped embryos. There is considerable evidence that the plant hormones abcisic acid (ABA) and gibberellins (GAs) are involved in the dormancy process. Whereas ABA is involved in regulating the onset of dormancy and in maintaining the dormant state (Bewley, 1997), GAs appear not to be involved in the control of

dormancy per se but rather are important in the promotion and maintenance of germination. An integrated view of seed dormancy and the control of germination can be found in Finch-Savage and Leubner-Metzger (2006).

Poor seed germination rate limits the use of different species for culture or plant breeding, and different treatments such us scarification, stratification and addition of different chemical substances are commonly used to promote germination in several species (Yoshioka et al., 1998; Bone, 2003; Brady and McCourt, 2003). Also dormancy mechanisms have been eliminated of some cultivated seed by selection and breeding.

In the genus *Solanum*, dormancy and low germination rates have been described in different species (Adebola and Afolayan, 2006; Taab and Andersson, 2009) including some accessions of *Solanum melongena* and related species (Joshua, 1978; Ibrahim et al., 2001; Demir et al., 2005). Thus, seeds of a number of locally cultivated *Solanum* species are known to emerge slowly, and about 30 days could be needed to attain germination with percentage rates between 15 and 50% in *S. incanum*, *S. torvum*, *S. integrifolium*, *S. surattense*, *S. khasianum*, *S. sanitwongsei* and hybrids of *S. melongena* x *S. ingrifolium* (Ibrahim et al., 2001). Scarce germination and low uniformity in percentage rates has been also described in *S. incanum* by Joshua (1978).

In this work we have studied seed germination and also applied several treatments for improving germination in eggplant and the related species *S. aethiopicum*, *S. incanum* and *S. macrocarpon*. These species are interesting in breeding as they display a wide variability (Daunay, 2008). Also, some accessions of these species showed resistance to several soil-borne pathogens and/or tolerance to drought (Gisbert et al., 2006; Lester et al., 1986), making them potentially useful rootstocks for common eggplant or other compatible crop species (Porcelli et al., 1990). Study and promotion of germination in these accessions is interesting for their use for breeding purposes and their potential use as rootstocks. Germination studies are also interesting for conservation and use of germplasm resources.

MATERIALS AND METHODS

Two accessions of the *Solanum* species *S. aethiopicum* (BBS-107, BBS-116), *S. macrocarpon* (BBS-117, BBS-168) and *S. incanum* (MM-557, MM-677); two *S. melongena* cultivars, Black Beauty (BB) and LF3 and the experimental hybrid *S. incanum* x *S. melongena* (MM-557 x ANS-26) were used in our experiments. All the seeds were maintained in dry storage for two years.

In a first experiment, seed germination rates were compared among the different *Solanum* accessions sown directly in Petri dishes (90×25 mm) with humid filter paper (HFP) or surface sterilized and shown in similar Petri dishes that also contained 40 ml of sterile nutrient medium (NM) alone or with 1 mg L^{-1} of GA_3 (NM GA_3). Seeds were surface-sterilized by inmersion for 10 min in a solution of 25% commercial bleach as described in Gisbert et al. (2006). The NM composition was: Murashige and Skoog (1962) salts including vitamins, 2% sucrose and 0.6% plant agar. The pH of the medium was adjusted to 5.8 with KOH before autoclaving at 121°C for 20 min. GA_3 was added to sterilized medium after filtration with sterile filters (200 µm).

In a second experiment, seeds of *S. incanum* and *S. macrocarpon* accessions were stratified at 4°C for 15 and 30 days and then sown in Petri dishes with NM and NM with GA_3.

In a third experiment, surface sterilized seeds of *S. aethiopicum* BBS-107, *S. macrocarpon* (BBS-168 and BBS-117), *S. incanum* (MM-557 and MM-667), the experimental hybrid MM-557 x ANS-26 and *S. melongena* LF3 were sown in NM and in NM supplemented with 1 mg L^{-1} GA_3 (GA_3), 0.3 mg L^{-1} Fluridone (F) or both (F+GA_3). All these components were additioned previous sterilization by filtration to sterilized NM medium. Seeds of BBS-168 were also sown in NM supplemented with 2 mg L^{-1} GA_3, 1 mg L^{-1} thidiazuron (TDZ) and 1 mg L^{-1} 6-bencylaminopurine (BAP).

In all these assays, 20 seeds per accession distributed in two Petri dishes were sown per treatment and maintained in a growth chamber at 25±2°C under a 16 h photoperiod with cool white light provided by fluorescent lamps (90 µmol m^{-2} s^{-1}).

RESULTS

A great variability for germination capacity in the different accessions of *S. melongena* and the related species assayed has been observed (Table 1). *S. melongena* and *S. aethiopicum* accessions had better germination rates than *S. macrocarpon* and *S. incanum* species, which had low or no germination. Thus, percentages of germination at 20 d after sowing in Petri dishes in the common germination treatment (HFP) ranged from 0% in *S. macrocarpon* BBS-117 and *S. incanum* MM-557 to 60% in *S. melongena* BB. Sterilized seeds sown on plates with NM had higher germination rates than those sown in HFP in all accessions with germinated seeds (Table 1). Addition of GA_3 to NM increased the germination rate in particular in *S. macrocarpon* BBS-168. Global mean percentages for HFP, NM and NM with GA_3 were 23.84, 55.70 and 70.38%, respectively. The addition of GA_3 to NM medium also reduced mean germination time. Thus, in this experiment radicles and hypocotyls could be observed emerging 7 d post-sowing from some *S. melongena* and *S. aethiopicum* seeds in NM plus GA_3. No seed of these accessions had germinated at this time (7 d post-sowing) in NM plates. A similar effect was observed in *S. macrocarpon* BBS-168, with 50% of seeds germinated in GA_3 containing medium and no germination in NM 9 d after sowing (Fig. 1). Stratification of *S. incanum* and *S. macrocarpon* seeds at 4°C for 15 and 30 d did not improve germination rates, which were similar to those obtained in the first experiment.

The use of Fluridone, an inhibitor of carotenoid biosynthesis that is known to prevent ABA biosynthesis, was tested in a third experiment. Germination rates were compared in plates with NM plus F, NM, NM supplemented with GA_3 and NM with both F and GA_3. Results 13 d after sowing were shown in Figure 2. All components added to NM promoted germination in all accessions tested with the exception of *S. incanum* MM-557 that did not germinate, as occurred in the previous assay. In general, the addition of F had a similar effect to that produced by GA_3. A slight synergistic effect of both added components was observed only in plates of *S. macrocarpon* BBS-117 and *S. melongena* LF3 (Fig. 2). The experimental hybrid MM-557 x ANS-26, derived from the MM-557 accession of *S. incanum* and the ANS-26 *S. melongena* accession, showed good germination rates (in average 70%) in NM medium supplemented with GA_3, F or both (Fig. 2).

Seeds of BBS-168 sown in NM supplemented with 1 mg L^{-1} of TDZ and BAP, and with GA_3 at 2 mg L^{-1} had germination rates of 30, 25 and 60%, respectively. Thus, TDZ and BAP did not increase germination in this accession, which had 35% of germination in NM. The addition of 2 mg L^{-1} of GA_3 had similar effect than 1 mg L^{-1} GA_3. Some plantlets from plates with BAP showed swelled radicles and hypocotyls. Also in TDZ some seeds gave abnormal plantlets.

DISCUSSION

A great variability for germination capacity in the different accessions of *S. melongena* and the related species *S. aethiopicum*, *S. incanum* and *S. macrocarpon* has been observed in these assays. In general, germination in *S. melongena* and *S. aethiopicum* is better than in *S. macrocarpon* and *S. incanum*, which had low or no germination. Dormancy, loss of viability during extraction or conservation conditions, genetic differences among the materials, aging or combination of these factors could explain these differences.

In order to increase germination rates several treatments have been applied. Sterilization and germination in NM resulted in higher germination rates than in HFP, the most common germination procedure in our laboratory to test seed germination. This promotion of germination could be the result of scarification and/or hydration suffered in the sterilization procedure of seeds, and/or of a better nutrient uptake. Soaking seeds in water was found to increase the germination rate in *Solanum aculastrum* (Adebola and Afolayan, 2006). Sterilization of seeds and culture on NM medium in vitro is laborious but permits to test the effect of several compounds, homogeneously added, and to follow the germination process in sterile conditions during a long period if necessary. In this work, several compounds were added after filtration.

The addition of GA_3 to culture medium has significantly enhanced germination in *S. macrocarpon* BBS-168 and, in general, in the rest of the treated accessions. There is considerable evidence that GAs are important in the promotion and maintenance of germination, and the application of GA_3 was effective in breaking dormancy in different plants including trees as *Myrica rubra* (Chen et al., 2008); weeds as leafy spurge (Foley and Chao, 2008), etc. In several species of *Solanum*, GA_3 has been used to promote germination with different results. Thus, Joshua et al. (1978) reported a higher efficiency of the treatment with GA_3 (GA, 500 ppm for 6 day) for promoting germination in *S. incanum* compared with decoated seeds, and the use of alternating temperatures (10/25°C). However, only a germination increase among 10-34% was produced in 3 of the 8 *Solanum* species treated with a GA_3 solution (100 mg L^{-1} in water for 24 h) by Ibrahim et al. (2001). We have also observed that seeds sown in GA_3 containing medium germinated before than those in NM. This effect has been observed in all *S. melongena* and *S. aethiopicum* accessions tested, and also in *S. macrocarpon* BBS-168. In some species like *Santalum album* (Nikam and Barmukh, 2009) a reduction in mean germination time after GA_3 application has been reported.

Other treatments applied in order to promote germination of *S. macrocarpon* and *S. incanum* seeds were stratification at 4°C and addition of Fluridone to NM medium. These treatments were used to promote seed germination in some species. For instance, Fluridone has restored the seed germination of some *Lactuca sativa* L. cultivars (Yoshioka et al., 1998). In this work, the temperature or time applied in the stratification treatment has not modified germination rates, but the addition of 0.3 mg L^{-1} F increased germination in both accessions of *S. macrocarpon*, in the hybrid from *S. incanum* x *S. melongena* and in the accessions of *S. aethiopicum* and *S. melongena* included in this experiment. This result indicates that, probably, ABA is limiting germination in these *Solanum* seeds. Comparation of germination rates in plates with GA_3, F or both indicate that F promotes germination with a similar range to that of GA_3. A slight synergistic effect F- GA_3 was observed in plates of *S. macrocarpon* BBS-117 and *S. melongena* LF3.

BAP has been used in in vitro embryo germination in aged almond (San and Yildirim, 2009) and also enhanced germination in *Sorghum bicolor* (Tiryaki and Buyukcingil, 2009). In our work, the addition of 1 mg L^{-1} of BAP or TDZ to NM medium had no possitive effect in germination of *S. macrocarpon* BBS-168 seeds.

None of assayed treatments has promoted *S. incanum* MM-557 germination, which could have lost viability or may suffer seasonal changes in seed dormancy as has been described in *Solanum nigrum* and *Solanum physalifolium* by Taab and Andersson (2009), who explained the late emergence of the species by a short-lived dormancy induction.

In conclusion, dormancy and germination are complex traits, and under similar storage conditions great differences for germination rate have been observed in the assayed accessions of *S. melongena* and some related species. Of the assayed treatments, sterilization of seeds and sowing in NM sterile medium with GA_3, Fluridone or both are the best for promoting germination in most of the assayed accessions.

ACKNOWLEDGEMENTS

This contribution was financed by the "Ministerio de Ciencia e Innovación" grants AGL2006-04878/AGR, AGL2009-07257, and RF2008-00008-00-00.

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Tables

Table 1. Final germination percentages in humid filter paper (HFP), nutrient medium (NM) and NM with 1 mg L^{-1} GA_3 (NM-GA_3).

	HFP	NM	NM-GA_3
S. melongena BB	60	95	100
S. melongena LF3	40	85	90
S. aethiopicum BBS-116	30	80	95
S. aethiopicum BBS-107	40	80	85
S. macrocarpon BBS-168	15	30	60
S. macrocarpon BBS-117	0	0	2
S. incanum MM-557	0	0	0
S. incanum MM-657	2	6	6
Global mean[a]	23.84 c	55.70 b	70.38 a

[a]Global means separated by different letters are significantly different ($P<0.05$) according to the Tukey test.

Figures

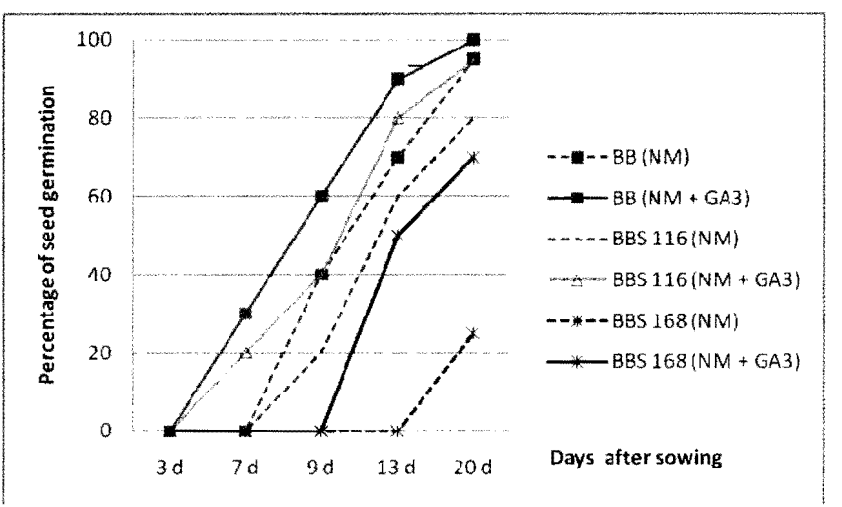

Fig. 1. Seed germination time courses of *S. melongena* (BB), *S. aethiopicum* (BBS 116) and *S. macrocarpon* (BBS 168) sown in Petri dishes with NM (---) and NM with GA_3 (–) at 3, 7, 9, 13 and 20 d after sowing.

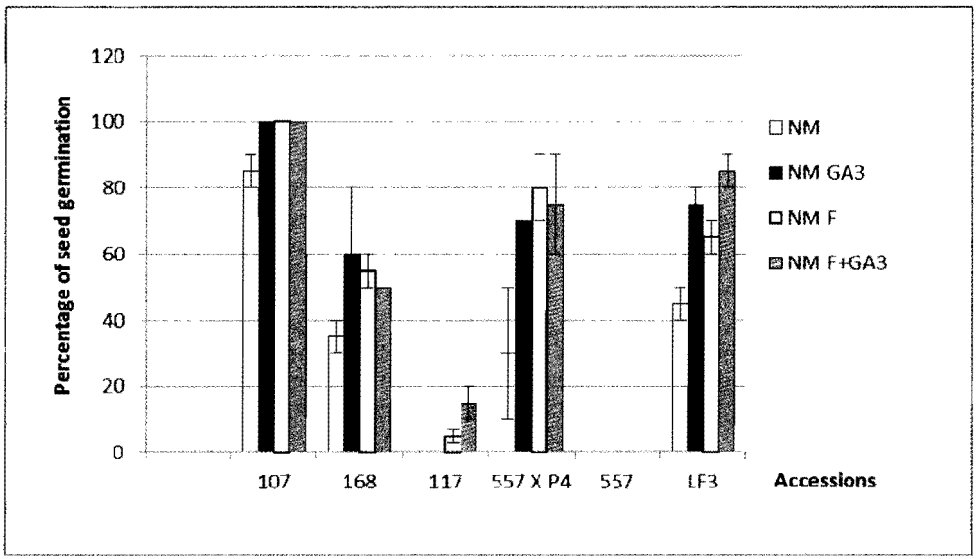

Fig. 2. Percentages of seed germination 13 d after sowing in NM and NM with GA_3 (1 mg L^{-1}), F (0.3 mg L^{-1}) and F + GA_3. Bars represent ± SE.